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EFFECTS OF TRIAMINOGUANIDINE NITRATE ON PREGNANT RATS

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

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Pregnant rats were dosed on gestation days 6 through 15 with either 0, 200, 400, or 800 mg TAGN/kg. Maternal weight loss occurred in the higher dose groups. At 800 mg/kg most litters were entirely resorbed. The lower dose groups exhibited an increased incidence of runting and perinatal death. An increase in malformations due to TAGN exposure was not observed.

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PREFACE

This research was performed in the Toxicology Branch, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory, from March 1980 through December 1980. It was performed in support of Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations;" Task 630201, "Toxicology of Conventional Propellants, Industrial Chemicals, and Materials;" Work Unit 63020104, "Teratogenic Screening of Air Force Chemicals."

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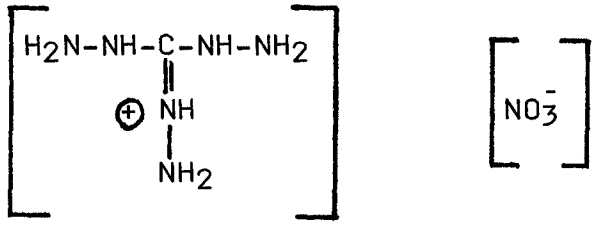
INTRODUCTION

In the Air Force, and other services as well, women now occupy a variety of jobs that were formerly considered the sole province of men. The majority of these women are in age groups of high fecundity and some of them are likely to be in the work environment while pregnant. Exposure of pregnant women to environmental chemicals may pose significant health hazards to both the mother and the fetus. Effects on the fetus include direct toxicity on the formed tissues and chemically-induced alterations in development leading to congenital defects. Congenital abnormalities caused by chemical exposure are most usually associated with exposure during the early part of pregnancy when most women would still be working. The study of congenital malformations is referred to as teratology and chemical teratogenesis is the production of malformations by exposure of pregnant animals to chemical agents. Any complete, contemporary evaluation of the toxicity of AF chemicals must include examination of their potential teratogenicity. This is especially true for materials with demonstrated ability to alter genetic information such as chemical mutagens and carcinogens.

Triaminoguanidine nitrate (TAGN), a candidate high energy propellant proposed for use within the AF, has the following chemical composition:



Davis et al. (1977) found that the acute ip LD_{50} for TAGN in the mouse was 3.7 g/kg and that TAGN caused a bradycardia in the dog at iv levels above 50 mg/kg. They concluded that the hazard from acute TAGN exposure was relatively low. Brusick and Matheson (1978) studied the mutagenic and oncogenic properties of TAGN. They found TAGN to be positive in several mutagen tests including the Ames salmonella microsome assay, the mouse lymphoma cell assay, and the unscheduled DNA synthesis assay. TAGN was negative in the dominant lethal assay in both the rat and the mouse. Brusick and Matheson concluded that TAGN had a high level of probability of carcinogenic activity.

No information about the embryotoxicity of TAGN is available. However because TAGN has a structure similar to hydroxyurea (NH_2-$\overset{O}{\overset{\|}{C}}$-NHOH), a very potent teratogen, and was found to be positive in several mutagenic assays, it was considered to be a potential teratogen. The purpose of this study was to assess the teratogenic hazard of TAGN exposure.

METHODS

Virgin female Fischer 344 rats (Charles River Breeding Laboratories) were housed in plastic cages containing wood chip bedding in a room maintained at 70-76°F with a 12 hour light cycle. The rats received Purina Lab Chow and water ad libitum. The females were placed with fertile males of the same stock overnight and checked for presence of sperm by vaginal wash the next morning. The day on which sperm was found was designated day zero of pregnancy. The pregnant rats were weighed daily.

TAGN (Rockwell International, Rocketdyne Division, Canoga Park CA) was diluted with distilled water and administered by intraperitoneal injections at doses of 200, 400, or 800 mg/kg daily on days 6 through 15 of gestation. A control group was also injected with an equivalent volume of distilled water.

The pregnant females were euthanized and the fetuses were delivered by caesarian section. The number and placement of fetuses and resorption sites were recorded. Fetuses were removed, weighed, sexed, and examined for external abnormalities. About 50% of each litter was fixed in Bouin's solution and the remainder in absolute ethanol. Fetuses fixed in Bouin's solution were serially sectioned with a razor blade and examined under a dissecting microscope for soft tissue abnormalities. Fetuses in ethanol were cleared in KOH, stained with Alizarin Red S and examined for skeletal defects (Olson and Back, 1978; Wilson and Warkany, 1965).

The means from measured data (body weights, implantation sites, viable fetuses, and resorptions) were analyzed for statistical significance by the Students-t method. Fetal abnormality data were analyzed for statistical significance by the Chi square test with the Yates modification.

RESULTS

There was a significant maternal weight deficit found in the two higher dose groups (Table 1). In the highest dose group (800 mg/kg) three females died during the dosing regimen, and only one viable litter was obtained. Most litter parameters in the two lower dose groups were not different from the control group (Table 1). Viable fetuses per litter and percent litters with resorptions were similar to those of the control, while resorptions per litter with resorptions increased slightly in the two lower dose groups when compared to the control. A significant increase in resorptions, and decrease in viable fetuses were found in the high dose group. Mean fetal body weight for both male and female fetuses was not different from the controls in either of the two low dose groups (Table 1).

TABLE 1

EFFECT OF TAGN ON MATERNAL, LITTER, AND FETAL PARAMETERS

PARAMETER	TAGN Dose (mg/kg)[1] 0	200	400	800[3,7]
Number of litters (fetuses)	12 (108)	14 (136)	10 (95)	8 (5)
Implantation sites/dam[2]	9.7 ± 0.7	10.6 ± 0.4	10.8 ± 0.4	8.8 ± 0.5
Viable fetuses/litter[2]	9.0 ± 0.9	9.0 ± 0.5	9.6 ± 0.7	.71 ± 0.7[4]
% Litters with resorptions	42	50	50	100
Resorptions/Litter with resorptions[2]	1.6 ± 0.4	2.3 ± 0.6	2.4 ± 0.5	8.1 ± 1.0[4,6]
Male fetus weight[2]	3.1 ± 0.03	3.0 ± 0.05	3.1 ± 0.04	2.8
Female fetus weight[2]	3.0 ± 0.03	2.9 ± 0.04	2.9 ± 0.05	2.7
Maternal weight gain[2,5]	26.4 ± 2.1	20.5 ± 3.3	1.6 ± 1.4[4]	-8.4 ± 1.2[4]

1. Intraperitoneal injection given on days 6-15 of gestation.
2. Mean ± S.E.
3. Only one viable litter (5 fetuses) was obtained.
4. Significantly different from control ($p \leq .05$).
5. Day 20 maternal weight with gravid uterus removed minus Day 0 maternal weight.
6. All resorptions were very early.
7. Three of eleven dams died during the TAGN dose regimen.

The incidence of fetal abnormalities was low in all dose groups (Table 2). Two of the control litters contained abnormal fetuses, while one litter in the 400 mg/kg and two litters in the 200 mg/kg groups contained abnormal fetuses. None of the abnormalities can be considered severe. The incidence of very small fetuses (runts), perinatal deaths, and late resorptions was much greater in the 200 mg/kg and 400 gm/kg groups than in the control group. The time of fetal resorption in the low dose groups was usually late gestation as opposed to the time of resorption in the high dose group which was early gestation. The high dose dams usually had very small metrial glands and no intrauterine material, while in many of the late resorptions in the low dose groups large fetal masses with discernible gross features were present. No dead fetuses were present in the control group while several were present in the 200 mg/kg group (Table 2).

Two dams given 600 mg/kg TAGN on days 6 through 15 of pregnancy gave results (100% early resorptions and marked maternal weight deficit) similar to those obtained from the 800 mg/kg TAGN group.

TABLE 2

EFFECT OF TAGN ON FETAL ABNORMALITIES

PARAMETER	TAGN Dose (mg/kg)[1] 0	200	400	800
Retained testicle	1	1	1	0
Rib agenisis	1	0	0	0
Bipartite vertebral centrae	0	1	0	0
Late resorptions and perinatal death	0	15	3	0
Runts:[2]				
Runts/total fetuses	2/108	15/136[3]	9/95[3]	0/8
Affected litters/total litters	2/12	4/14	5/10	---

1. Intraperitoneal injection given on days 6-15 of gestation.
2. Mean control fetus weight minus 3 S.D.
3. Significantly different from control ($p \leq .05$).

DISCUSSION

The results of this study indicate that the principal effect of TAGN on the rat fetus is one of toxicity. The effect is most obvious in the high dose group, where almost 100% of the fetuses were resorbed (Table 1). There was a sharp change in results between the high dose group (800 mg/kg) and the middle dose group (400 mg/kg) with respect to fetal toxicity. In the 800 mg/kg group almost 100% of the fetuses were resorbed very early in pregnancy, while the two lower dose groups had an increased incidence of late resorptions and perinatal death.

The high incidence of early resorptions coupled with the 3 of 11 dams that died in the 800 mg/kg dose group indicates a concurrent toxic effect on both the fetus and dam. It is possible that a local concentration effect on the fetuses was responsible for the high incidence of early resorptions in the 800 mg/kg TAGN group. The dams in the two higher dose groups (400 mg/kg and 800 mg/kg) were observed to exhibit apparent discomfort in the abdominal region immediately following ip injection. This was particularly severe in the 800 mg/kg group and was evidenced by a clonic contraction of the abdominal musculature for 10 to 15 seconds immediately following the ip injection. It is not known if this reaction was due to TAGN itself or to irritation from the injection of a concentrated solution with a pH of about 5. No gross pathology was observed in the area of the injections when the dams were examined following

caesarian section to remove the fetuses, or in the three dams that died during the injection regimen. Maternal toxicity was also exhibited as a weight deficit in the 800 mg/kg dose group as well as the 400 mg/kg dose group when compared to the control group (Table 1). Fetotoxicity was evident at the two lower doses in the form of growth retardation (runting) and perinatal death (Table 2).

While there was no consistent dose-related increase in the frequency of occurrence among fetuses of these observations as dose increased from 200 to 400 mg/kg/day, the numbers of runts and stillborn at these doses do appear to be significantly higher than for control. In addition, the number of litters affected did appear to be dose related (Table 2). There are two potential explanations for the lack of a consistent dose-response curve for this otherwise significant level of fetotoxicity. First, the complex dose-response pattern may be a composite produced by the superposition of two effects - fetotoxicity at lower doses coupled with overt maternal toxicity at the higher dose (see maternal weight gain in Table 1). Second, plateaus in dose-response curves have been reported for substances where metabolites are responsible for toxicity. Perhaps the fetotoxicity of TAGN is caused by some metabolite. Further experimentation is required to see if one of these explanations is valid, or if the effect is due to some other mechanism entirely.

Because of the sharp change in toxic effect noted between the 800 mg/kg and 400 mg/kg groups, it was decided to dose two dams with 600 mg/kg TAGN. The results were essentially identical to those obtained from the 800 mg/kg group.

The late resorptions, perinatal death, and growth retardation found in this study are generally regarded as fetotoxic rather than teratogenic effects. TAGN does not seem to be teratogenic in the rat. Assessment of the hazard from TAGN fetotoxicity is more complex. Some evidence for fetotoxicity has been presented, but the exact nature and degree of the hazard to man cannot be determined from these data. However, since TAGN is a crystalline solid, the probability of a significant fetal TAGN exposure via the usual occupational routes of concern (inhalation or percutaneous) seems slight.

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